

Two New Highly Oxygenated Spirostanol Saponins from *Paris polyphylla* var. *stenophylla*

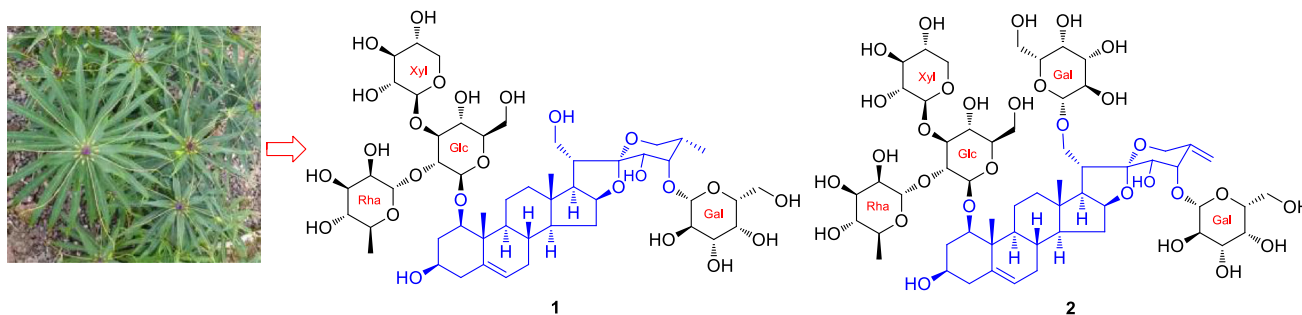


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Abstract Phytochemical investigation of the rhizomes of *Paris polyphylla* var. *stenophylla* led to the isolation of two new highly oxygenated spirostanol saponins, named paristenosides A (**1**) and B (**2**), together with seven known compounds. Their structures were established mainly on the base of NMR spectroscopic techniques and mass spectrometry, as well as chemical methods. In addition, the cytotoxicity of the two new saponins was tested.

Graphical Abstract Two new highly oxygenated spirostanol saponins, paristenosides A (**1**) and B (**2**), were isolated from the rhizomes of *Paris polyphylla* var. *stenophylla*. Their structures were established mainly based on NMR spectroscopic techniques and mass spectrometry, as well as chemical methods.



Keywords *Paris polyphylla* var. *stenophylla* · Liliaceae · Spirostanol saponins · Paristenoside A · Paristenoside B

Ling-Yu Jin and Ting-Xiang Lu contributed equally to this work.

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1 Introduction

The genus *Paris* (Liliaceae) comprises about 24 species, which grows as perennial rhizomatous herbs in the Eurasian continent from temperate zone to the tropics [1]. Rhizoma Paridis ('Chonglou' in Chinese), the dried rhizomes of *P. polyphylla* var. *yunnanensis* and *P. polyphylla* var. *chinensis*, have been recorded in the Pharmacopoeia of the People's Republic of China as a traditional Chinese medicine for the treatment of traumatic injuries, insect and snake bites, and sore throat [2]. Plants of the genus *Paris* are known as a rich source of steroidal saponins, which have attracted great interests for their structural diversity and various bioactivities such as anti-tumor [3, 4], hemostatic [5], and antifungal [6] effects. In recent years, our group focuses the research on the chemical constituents, bioactivity, and sustainable utilization of resources of the genus [7–11].

P. polyphylla var. *stenophylla* Franch. is one of the variations of *P. polyphylla* and has been used as a herbal medicine to treat skin furuncle and skin tinea, stop bleeding, and move clean blood and bad blood out by the Yi nationality of Liangshan in Sichuan Province [12]. Due to having certain resources, its rhizomes have been as the main steam commodities of Rhizoma Paridis. However, there are a few reports about the research of its material basis [13]. In order to clarify its chemical quality, we performed a phytochemical investigation of the rhizomes of *P. polyphylla* var. *stenophylla*. As a result, two new polyhydroxylated steroidal saponins, named paristenosides A (1) and B (2), were isolated from the title species. Meanwhile, seven known compounds were obtained and identified as paris saponin H (3) [14], Tg (4) [15], Pb (5) [16], Th (6) [17], Methyl-Th (7) [18], parispseudoside A (8) [19] and β -ecdysone (9) [16]. In the current paper, we report the isolation, structural elucidation, and cytotoxicity of the two new compounds (Fig. 1).

2 Results and Discussion

Paristenoside A (1) was isolated as a white amorphous powder. Its molecular formula, $C_{50}H_{80}O_{25}$, was assigned by the positive-ion HR-ESI-MS (m/z 1103.4881 $[M + Na]^+$, calcd for 1103.4881) and ^{13}C NMR data (Tables 1, 2). The IR spectrum displayed absorption bands ascribable to hydroxy (3426 cm^{-1}) and olefinic bond (1631 cm^{-1}) functionalities. The ^{13}C NMR spectrum showed signals for a ketal carbon at δ_C 111.5, a trisubstituted olefin carbons at δ_C 139.4 (s) and 124.6 (d), and three methyls at δ_C 17.0, 15.0, and 13.1, which were characteristic of a $\Delta^{5,6}$ -spirostanol skeleton as an aglycone [7]. Its 1H NMR spectrum showed two singlet signals for two tertiary methyls at δ_H 1.38 and 1.13, one doublet signal for a secondary methyl at δ_H 1.03 (d, $J = 6.8\text{ Hz}$), an olefinic proton at δ_H 5.55 (br d, $J = 5.5\text{ Hz}$), as well as four anomeric protons at δ_H 6.41 (br s), 5.24 (d, $J = 7.8\text{ Hz}$), 4.91 (d, $J = 7.6\text{ Hz}$), and 4.78 (d, $J = 7.7\text{ Hz}$), which suggested the presence of four sugar units. In addition, one methyl at δ_H 1.71 (d, $J = 6.0\text{ Hz}$) was the methyl of one 6-deoxyhexopyranose residue. Comparison of the 1H and ^{13}C NMR spectra of 1 with those of padelaoside A [20] revealed that 1 possessed the same polyhydroxylated aglycone [(23S,24S,25S)-spirost-5-ene-1 β ,3 β ,21,23,24-pentol] as that of padelaoside A, which could be further verified by detailed 2D NMR data analysis (Figs. 2, 3). Furthermore, the result of acid hydrolysis and GC analysis of 1 with its corresponding trimethylsilylated L-cysteine adducts afforded D-glucose, D-galactose, D-xylose, and L-rhamnose. The large coupling constants ($^3J_{1,2} > 7\text{ Hz}$) were consistent with β -configuration for the glucose, galactose, and xylose, while the α -configuration for the rhamnose was deduced by comparing its ^{13}C NMR spectroscopic data for C-3'' (δ_C 72.4) and C-5'' (δ_C 69.5) with those reported in the literature [21]. The sequence and binding sites of the sugar units were determined by following HMBC correlations: δ_H 6.41 (H-1'' of Rha) with δ_C

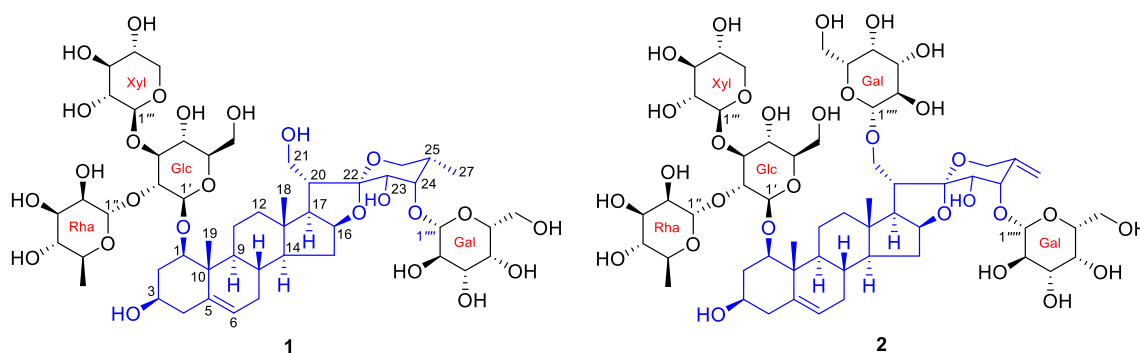


Fig. 1 Chemical structures of paristenosides A (1) and B (2)

Table 1 ^1H and ^{13}C NMR data for the aglycone portions of paristenosides A (**1**) and B (**2**) (in $\text{C}_5\text{D}_5\text{N}$, 600 MHz)

No.	Paritenoside A		Paritenoside B	
	δ_{C}	δ_{H} (mult., J in Hz) ^a	δ_{C}	δ_{H} (mult., J in Hz) ^a
1	83.9 (CH)	3.85 (dd, 12.0, 3.8)	84.6 (CH)	3.77 (dd, 11.9, 3.9)
2a	37.7 (CH ₂)	2.65	38.0 (CH ₂)	2.63
2b		2.38 (q, 11.9)		2.39 (q, 12.0)
3	67.9 (CH)	3.74 (m)	67.9 (CH)	3.73 (m)
4a	43.6 (CH ₂)	2.66 (br t, 12.2)	43.7 (CH ₂)	2.65 (t, 12.6)
4b		2.54 (dd, 11.6, 3.7)		2.52 (dd, 12.0, 3.5)
5	139.4 (C)		139.3 (C)	
6	124.6 (CH)	5.55 (br d, 5.5)	124.7 (CH)	5.52 (br d, 5.3)
7a	31.7 (CH ₂)	1.67 (m)	31.7 (CH ₂)	1.72 (m)
7b		1.40 (m)		1.43 (m)
8	33.0 (CH)	1.48 (m)	32.9 (CH)	1.45 (m)
9	50.0 (CH)	1.61 (m)	50.2 (CH)	1.59 (m)
10	42.6 (C)		42.6 (C)	
11a	24.0 (CH ₂)	2.82 (m)	23.9 (CH ₂)	2.77 (m)
11b		1.61 (m)		1.47 (m)
12a	40.2 (CH ₂)	1.92 (m)	39.9 (CH ₂)	1.88 (br d, 9.5)
12b		1.48		1.46
13	41.0 (C)		40.9 (C)	
14	56.9 (CH)	1.15 (m)	56.9 (CH)	1.14 (m)
15a	32.4 (CH ₂)	1.80 (m)	32.2 (CH ₂)	1.80 (m)
15b		1.47		1.43
16	83.1 (CH)	4.58	83.3 (CH)	4.53
17	58.1 (CH)	2.00 (t, 7.2)	58.1 (CH)	1.94 (t, 7.5)
18	17.0 (CH ₃)	1.13 (s)	16.8 (CH ₃)	1.03 (s)
19	15.0 (CH ₃)	1.38 (s)	14.9 (CH ₃)	1.32 (s)
20	46.0 (CH)	3.34 (br t, 5.6)	43.5 (CH)	3.42 (q, 6.7)
21a	62.5 (CH ₂)	4.20 (m)	69.8 (CH ₂)	4.43 (m)
21b		4.04 (m)		3.94 (m)
22	111.5 (C)		111.3 (C)	
23	71.1 (CH)	4.10 (m)	71.1 (CH)	4.34 (d, 2.6)
24	81.8 (CH)	4.17 (m)	82.0 (CH)	4.68 (d, 2.6)
25	35.1 (CH)	1.93 (m)	143.4 (C)	
26a	61.4 (CH ₂)	3.98 (m)	61.4 (CH ₂)	4.84 (d, 12.2)
26b		3.35 (m)		3.98 (d, 12.2)
27a	13.1 (CH ₃)	1.03 (d, 6.8)	113.8 (CH ₂)	5.04 (s)
27b				4.96 (s)

^a Overlapped signals are reported without designating multiplicity

76.3 (C-2' of Glc), δ_{H} 4.91 (H-1''' of Xyl) with δ_{C} 88.3 (C-3' of Glc), δ_{H} 4.78 (H-1' of Glc) with δ_{C} 83.9 (C-1 of the aglycone), and δ_{H} 5.24 (H-1''' of Gal) with δ_{C} 81.8 (C-24 of the aglycone). Assignment of all proton and carbon resonances was achieved by interpretation of ^1H - ^1H COSY, HSQC, and HMBC spectra. Based on the above information, the structure of paritenoside A (**1**) was elucidated as 24-*O*- β -D-galactopyranosyl-(23*S*,24*S*,25*S*)-spirost-5-ene-1 β ,

3 β ,21,23,24-pentol-1-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 2)-[β -D-xylopyranosyl-(1 \rightarrow 3)]- β -D-glucopyranoside.

Paritenoside B (**2**) was obtained as a white amorphous powder with a molecular formula of $\text{C}_{56}\text{H}_{88}\text{O}_{30}$, which was deduced from the positive HR-ESI-MS (m/z 1258.5689 [$\text{M} + \text{NH}_4$]⁺, calcd for 1258.5699) and ^{13}C NMR data (Tables 1, 2). The ^1H and ^{13}C NMR data suggested that compound **2** was also a highly oxygenated spirostanol

Table 2 ^1H and ^{13}C NMR data for the sugar moieties of paristenosides A (**1**) and B (**2**) (in $\text{C}_5\text{D}_5\text{N}$, 600 MHz)

No.	Paristenoside A		No.	Paristenoside B	
	δ_{C}	δ_{H} (mult., J in Hz) ^a		δ_{C}	δ_{H} (mult., J in Hz) ^a
1-Glc-1'	99.7 (CH)	4.78 (d, 7.7)	1-Glc-1'	100.2 (CH)	4.73 (d, 7.6)
2'	76.3 (CH)	4.13 (m)	2'	76.2 (CH)	4.06
3'	88.3 (CH)	4.03 (m)	3'	88.2 (CH)	4.01 (t, 9.2)
4'	70.1 (CH)	3.73 (m)	4'	69.8 (CH)	3.83 (t, 9.2)
5'	77.7 (CH)	3.77 (m)	5'	77.6 (CH)	3.74 (m)
6'a	63.1 (CH_2)	4.45 (m)	6'a	63.1 (CH_2)	4.45 (m)
6'b		4.14 (m)	6'b		4.22 (m)
2'-Rha-1''	101.6 (CH)	6.41 (br s)	2'-Rha-1''	101.6 (CH)	6.34 (br s)
2''	72.4 (CH)	4.75 (m)	2''	72.4 (CH)	4.75
3''	72.4 (CH)	4.57 (m)	3''	72.3 (CH)	4.55 (dd, 9.5, 3.3)
4''	74.1 (CH)	4.29 (m)	4''	74.0 (CH)	4.28 (t, 9.4)
5''	69.5 (CH)	4.81 (dq, 9.2, 6.3)	5''	69.4 (CH)	4.78 (dq, 9.2, 6.2)
6''	19.2 (CH_3)	1.71 (d, 6.0)	6''	19.1 (CH_3)	1.70 (d, 6.1)
3'-Xyl-1'''	105.1 (CH)	4.91 (d, 7.6)	3'-Xyl-1'''	105.1 (CH)	4.90 (d, 7.7)
2'''	74.6 (CH)	3.95 (t, 8.1)	2'''	74.6 (CH)	3.92 (t, 8.2)
3'''	78.3 (CH)	4.06 (t, 8.9)	3'''	78.3 (CH)	4.05
4'''	70.5 (CH)	4.10 (m)	4'''	70.5 (CH)	4.08 (m)
5'''a	67.1 (CH_2)	4.23 (dd, 11.3, 5.0)	5'''a	67.1 (CH_2)	4.23 (m)
5'''b		3.66 (t, 10.6)	5'''b		3.66 (t, 10.8)
24-Gal-1''''	106.3 (CH)	5.24 (d, 7.8)	21-Gal-1''''	105.1 (CH)	4.85 (d, 7.5)
2''''	72.4 (CH)	4.56	2''''	72.3 (CH)	4.44
3''''	75.3 (CH)	4.14 (m)	3''''	75.3 (CH)	4.12 (dd, 9.4, 3.2)
4''''	70.0 (CH)	4.53 (m)	4''''	70.1 (CH)	4.52
5''''	76.9 (CH)	4.06 (br t, 8.8)	5''''	76.9 (CH)	4.06
6''''a	62.0 (CH_2)	4.47 (m)	6''''	62.2 (CH_2)	4.38 (2H, br d, 5.9)
6''''b		4.37 (m)	24-Gal-1''''	103.9 (CH)	5.82 (d, 8.2)
			2''''	70.7 (CH)	4.64 (dd, 8.1, 2.9)
			3''''	75.2 (CH)	4.68 (m)
			4''''	71.1 (CH)	4.59 (br d, 3.2)
			5''''	76.9 (CH)	4.06
			6''''	62.3 (CH_2)	4.31 (2H, dd, 11.0, 5.7)

^a Overlapped signals are reported without designating multiplicity

glycoside. A comparison of the NMR spectra of **2** with those of parisyunnanoside I [22] disclosed that they both had the same aglycone and sugar chain linkages at C-1 and C-21, except for the replacement of the fucose by a galactose at C-24 in the latter. This was further supported by the HMBC correlation between δ_{H} 5.82 (H-1'''' of Gal) with δ_{C} 82.0 (C-24 of the aglycone). The other parts of **2** were identical to those of parisyunnanoside I as confirmed by detailed analysis of its 2D NMR experiments. Therefore, the structure of paristenoside B (**2**) was established as 21-*O*- β -D-galactopyranosyl-24-*O*- β -D-galactopyranosyl-(23*S*, 24*S*)-spirost-5,25(27)-diene-1 β ,3 β ,21,23,24-pentol-1-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 2)-[β -D-xylopyranosyl-(1 \rightarrow 3)]- β -D-glucopyranoside.

The phytochemical investigation of the rhizomes of *P. polyphylla* var. *stenophylla* led to the identification of eight steroidal saponins and one ecdysone, including two new highly oxygenated spirostanol glycosides. Compounds **1** and **2** are a further addition to the diverse and complex steroidal saponins. Our results and the literature [13] suggested that *P. polyphylla* var. *stenophylla* contained the same bioactive components as those in the legally original plants of *Rhizoma Paridis*. However, whether the rhizomes of *P. polyphylla* var. *stenophylla* can be used as the original plant of *Rhizoma Paridis* are still valuable for us to further research. Considering cytotoxicity of steroidal glycosides, paristenosides A (**1**) and B (**2**) were evaluated for their cytotoxicity against two human cancer cell lines (HEK293 and HepG2)

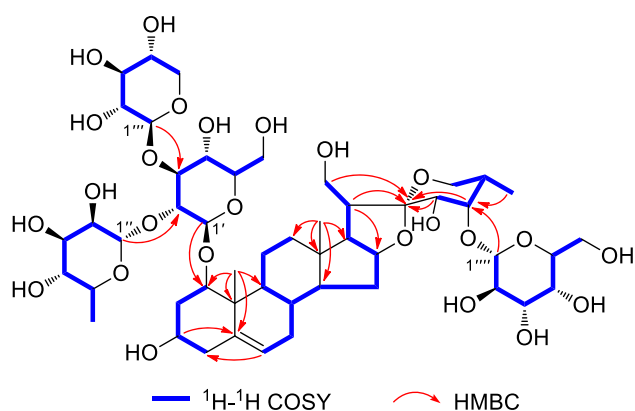


Fig. 2 ^1H - ^1H COSY and Key HMBC correlations of paristenoside A (**1**)

by using MTT method. Unfortunately, none of them showed significant activity at the concentration of 20 μM .

3 Experimental Section

3.1 General Experimental Procedures

Optical rotations were recorded on a JASCO P-1020 digital polarimeter. IR spectra were obtained on Bruker Tensor-27 infrared spectrophotometer with KBr pellets. ESI-MS spectra were recorded on a Bruker HCT/Esquire spectrometer. HR-ESI-MS were obtained on an Agilent 6200 Q-TOF MS system. 1D and 2D NMR spectra were obtained on a Bruker Avance III 600 MHz spectrometer in $\text{C}_5\text{D}_5\text{N}$; chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Puke Chemical Co. Ltd., China) and Rp-18 gel (40–63 μm , Merck, Darmstadt, Germany). GC analysis was performed on a HP5890 gas chromatograph equipped with an H_2 flame ionization detector. Semi-preparative HPLC was run on Agilent 1100 liquid chromatograph equipped with a Zorbax SB-C18 column (5 μm , 25 cm \times 9.4 mm) and a diode array

detector (DAD). TLC was performed on HSGF₂₅₄ (0.2 mm, Qingdao Puke Chemical Co. Ltd., China). Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10 % H_2SO_4 in EtOH.

3.2 Plant Material

The rhizomes of *P. polyphylla* var. *stenophylla* were collected in October 2014 from Zhaoyang District, Zhaotong, Yunnan province, China. The plant material was authenticated by Dr. Yun-Heng Ji, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. HY0023) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and Isolation

The air-dried and powdered rhizomes of *P. polyphylla* var. *stenophylla* (370 g) were extracted three times with 75 % EtOH under reflux and then the solvent was evaporated under reduced pressure to afford a crude extract (10 g). The crude extract was subjected to a silica gel column and eluted with a CHCl_3 –MeOH (15:1 \rightarrow 1:2, v/v) gradient solvent system to yield seven fractions (A–G). Fr. C (1.1 g) was chromatographed through a silica gel column (CHCl_3 –MeOH, 15:1 \rightarrow 1:1) and purified by semi-prep. HPLC (CH_3CN – H_2O , 40:60, v/v) to yield **3** (35 mg). Fr. E (1.5 g) was separated by an Rp-18 column (CH_3OH – H_2O , 50:50 \rightarrow 70:30, v/v) to get **4** (9 mg) and **5** (30 mg). Fr. F (1.5 g) was separated by a silica gel column, eluting with CHCl_3 –MeOH (10:1 \rightarrow 1:1) and was further purified by semi-prep. HPLC with the mobile phase of CH_3CN – H_2O (30:70, v/v) to obtain **1** (9 mg), **2** (5 mg), **6** (43 mg), **7** (50 mg), **8** (5 mg), and **9** (9 mg).

3.3.1 Paristenoside A (**1**)

White amorphous powder; $[\alpha]_{\text{D}}^{23}$ –76.8 (c = 0.05, MeOH); IR (KBr) ν_{max} : 3426, 1631, 1383, 1051 cm^{-1} ; ^1H

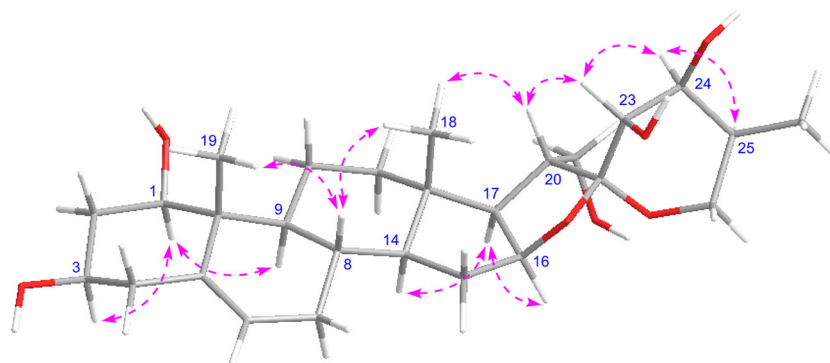


Fig. 3 Key ROESY correlations for the aglycone moiety of paristenoside A (**1**)

(600 MHz, C_5D_5N) and ^{13}C NMR (150 MHz, C_5D_5N) data, see Tables 1 and 2; ESIMS m/z 1103 $[M + Na]^+$; HRESIMS m/z 1103.4881 $[M + Na]^+$ (calcd for $C_{50}H_{80}O_{25}Na$, 1103.4881).

3.3.2 Paristenoside B (2)

White amorphous powder; $[\alpha]_D^{23}$ -55.8 ($c = 0.09$, MeOH); IR (KBr) ν_{max} : 3424, 1635, 1382, 1045 cm^{-1} ; 1H (600 MHz, C_5D_5N) and ^{13}C NMR (150 MHz, C_5D_5N) data, see Tables 1 and 2; ESIMS m/z 1263 $[M + Na]^+$; HRESIMS m/z 1258.5689 $[M + NH_4]^+$ (calcd for $C_{56}H_{92}O_{30}N$, 1258.5699).

3.4 Sugar Analysis of 1 and 2

Compounds 1 and 2 (each 2 mg) were refluxed with 2 M HCl (1,4-dioxane/ H_2O 1:1, 2 mL) on water bath for 2 h. The mixture was concentrated in a vacuum, and the residue was suspended in H_2O and then extracted with $CHCl_3$ (5 mL \times 3). The aqueous layer was neutralized with MeOH and then dried to give a mixture of sugars. Each mixture was dissolved in anhydrous pyridine (1 mL) and reacted with L-cysteine methyl ester hydrochloride (1.5 mg) stirred at 60 °C for 1.5 h. The trimethylsilylimidazole (1.0 mL) was subsequently added to the reaction mixtures, and they were kept at 60 °C for 30 min. The mixture (4 μ L) was finally analyzed by an HP 5890 gas chromatograph with a 30QC2/AC-5 quartz capillary column (30 mm \times 0.32 mm \times 0.25 μ m), respectively, under the following conditions: H_2 flame ionization detector; carrier gas: N_2 (1 mL/min); column temperature program: 180–280 °C with the rate of 3 °C/min; injector temperature: 250 °C; split ratio 1:50. Peaks of the hydrolysate were detected by comparison with retention times of authentic samples of D-glucose, D-galactose, D-xylose, and L-rhamnose after the aforementioned treatment in pyridine. The absolute configurations of the sugar moieties were identified as D-glucose ($t_R = 19.0$ min), D-galactose ($t_R = 23.0$ min), D-xylose ($t_R = 18.3$ min), and L-rhamnose ($t_R = 15.4$ min), respectively.

3.5 Cytotoxic Assay

Cytotoxic evaluations were performed for two human cancer cell lines (HEK293 and HepG2) using the MTT method described in the literature elsewhere [10]. (–)-OddC was used as the positive control and showed IC_{50} values for the two cancer cell lines with IC_{50} values of 0.30 and 0.17 μ M, respectively. The experiments were conducted for three independent replicates, and $IC_{50} > 20$ μ M was considered to be inactive.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

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References

1. H. Li, *The Genus Paris* (Science Press, Beijing, 1998), pp. 12–16
2. Chinese Pharmacopoeia Commission, *Pharmacopoeia of the People's Republic of China*, vol. 1 (Chinese Medical Science and Technology Press, Beijing, 2015), pp. 243–244
3. Z.X. Cheng, B.R. Liu, X.P. Qian, Y.T. Ding, W.J. Hu, J. Sun, L.X. Yu, *J. Ethnopharmacol.* **120**, 129–137 (2008)
4. S.L. Man, W.Y. Gao, Y.J. Zhang, L.L. Yan, C.Y. Ma, C.X. Liu, L.Q. Huang, *Steroids* **74**, 1051–1056 (2009)
5. Y.L. Fu, Z.Y. Yu, X.M. Tang, Y. Zhao, X.L. Yuan, S. Wang, B.P. Ma, Y.W. Cong, *J. Thromb. Haemost.* **6**, 524–533 (2007)
6. D.W. Deng, D.R. Lauren, J.M. Cooney, D.J. Jensen, K.V. Wurms, J.E. Upritchard, R.D. Cannon, M.Z. Wang, M.Z. Li, *Planta Med.* **74**, 1397–1402 (2008)
7. X.J. Qin, D.J. Sun, W. Ni, C.X. Chen, Y. Hua, L. He, H.Y. Liu, *Steroids* **77**, 1242–1248 (2012)
8. X.J. Qin, C.X. Chen, W. Ni, H. Yan, H.Y. Liu, *Fitoterapia* **84**, 248–251 (2013)
9. C.L. Sun, W. Ni, H. Yan, Z.H. Liu, L. Yang, Y.A. Si, Y. Hua, C.X. Chen, L. He, J.H. Zhao, H.Y. Liu, *Steroids* **92**, 90–95 (2014)
10. Y.S. Wen, W. Ni, X.J. Qin, H. Yan, C.X. Chen, Y. Hua, Y.C. Cheng, L. He, H.Y. Liu, *Phytochem. Lett.* **12**, 31–34 (2015)
11. X.J. Qin, M.Y. Yu, W. Ni, H. Yan, C.X. Chen, Y.C. Cheng, L. He, H.Y. Liu, *Phytochemistry* **121**, 20–29 (2016)
12. H.X. Yin, F.Y. Wen, H. Zhang, *World Sci Technol. Mod. Trad. Chin. Med. Mat. Med.* **16**, 177–180 (2014)
13. H.X. Yin, D. Xue, N. Bai, C. Chen, Y. Chen, H. Zhang, *J. Sichuan Univ. (Med. Sci. Ed.)* **39**, 485–488 (2008)
14. C.X. Chen, J. Zhou, Y.T. Zhang, C.K. Gao, *Acta Bot. Yunnan.* **5**, 219–223 (1983)
15. C.X. Chen, Y.T. Zhang, J. Zhou, *Acta Bot. Yunnan.* **5**, 91–97 (1983)
16. C.X. Chen, J. Zhou, *Acta Bot. Yunnan.* **3**, 89–93 (1981)
17. T. Nohara, K. Migahara, T. Kawasaki, *Chem. Pharm. Bull.* **23**, 872–885 (1975)
18. K. Nakano, K. Murakami, T. Nohara, T. Tomimatsu, T. Kawasaki, *Chem. Pharm. Bull.* **29**, 1445–1451 (1981)
19. C.M. Xiao, J. Huang, X.M. Zhong, X.Y. Tan, P.C. Deng, *Helv. Chim. Acta* **92**, 2587–2595 (2009)
20. T. Zhang, H. Liu, X.T. Liu, X.Q. Chen, Q. Wang, *Steroids* **74**, 809–813 (2009)
21. R. Kasai, M. Okihara, J. Asakawa, K. Mizutani, O. Tanaka, *Tetrahedron* **35**, 1427–1432 (1979)
22. L.P. Kang, Y.X. Liu, T. Eichhorn, E. Daput, H.S. Yu, Y. Zhao, C.Q. Xiong, C. Liu, T. Efferth, B.P. Ma, *J. Nat. Prod.* **75**, 1201–1205 (2012)